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Abstracts

Fertilization

Program/Abstract # 280

Live imaging analysis of mouse sperm acrosomal exocytosis

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Sperm acrosomal exocytosis is essential for successful fertilization, and the zona pellucida (ZP) has been classically considered as the primary initiator in vivo. At present, following what is referred to as primary binding, the acrosome reaction paradigm posits that the OAM and PMfuse, releasing the contents of the acrosome in a random manner. It is then assumed that secondary binding of the sperm to the ZP is mediated by the inner acrosomal membrane. In the present work using a live-imaging system and mice expressing GFP in the sperm acrosomes (GFP expression and targeting is driven by the proacrosin promoter and signal sequence), we compared the process of acrosomal exocytosis stimulated by the calcium ionophore ionomycin or acid-solubilized ZP. Surprisingly, acrosomal exocytosis driven by these two agents followed different patterns. When ionomycin was used, exocytosis was rapid and started randomly (no preference for the anterior, central, orposterior acrosomal regions). However with ZP, even though this normally particulate extracellular matrix had been solubilized, exocytosis was slower and the loss of acrosomal components (as monitored by the disappearance of GFP fluorescence) always started at the anterior part of the head and progressed to the posterior of the acrosome. These results demonstrate that ZP stimulate sacrosomal exocytosis in an orderly manner and suggest that a receptor-mediated event controls this process of membrane fusion and release of acrosomal components. NIH support: HD-41552 and Fogarty 5D43TW000671.

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Program/Abstract # 281

TSSK6, a member of the testis-specific serine kinase family, is required for sperm-egg fusion in the mouse

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The Testis Specific Serine Kinases (TSSKs) are a family of proteins with a testis specific pattern of expression. Male mice null forTssk6, one member of this protein family, have been reported to be sterile. In the present work we analyze the factors that make these mice infertile. Sperm from the TSSK6 null mice were unable to fertilize eggs *in vitro* but were able to activate development by Intra Citoplasmic Sperm Injection. To further analyze this mechanism, Zonae Pellucidae-free eggs from Wild Type mice were used *in vitro*. In this facilitated condition, the Tssk6$^{-/-}$ sperm were able to attach to the egg oolema; however, the Tssk6$^{-/-}$ sperm were unable to fuse. IZUMO, a protein involved in sperm–egg fusion was found to be present at normal levels in the Tssk6$^{-/-}$ sperm and to immunolocalize to the sperm acrosome similarly to wild type sperm. However, the acrosome reaction-associated change in the IZUMO localization pattern was not observed in the null sperm. This discovery strongly suggests that changes in IZUMO immunoreactivity after the acrosome reaction are necessary for sperm–egg fusion. It also demonstrates that the acrosome reaction alone is not sufficient to cause IZUMO changes. We have also investigated potential mechanisms for IZUMO movements and the possibility that IZUMO is phosphorylated in sperm. Altogether, these results provide insights into the molecular mechanisms of sperm–egg fusion and the signal transduction cascades associated with it.

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Program/Abstract # 282

Sorbitol can fuel mouse sperm motility and protein tyrosine phosphorylation via sorbitol dehydrogenase

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Energy sources that can be metabolized to yield ATP are essential for normal sperm functions such as motility. Two major monosaccharides, sorbitol and fructose, are present in semen. Furthermore, sorbitol dehydrogenase (SORD) can convert sorbitol to fructose which can then be metabolized via the glycolytic pathway in sperm to make ATP. Here we characterized SORD during mouse spermatogenesis and in epididymal sperm and examined the ability of sorbitol to support sperm motility and tyrosine phosphorylation. *Sord* mRNA levels increased during the course of spermatogenic differentiation. SORD protein, however, was first detected at the condensing spermatid stage. By indirect immunofluorescence, SORD was present along the length of the flagella of caudal epididymal sperm. Furthermore, immunoelectron microscopy showed that SORD existed within

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